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REVIEW

Hepatic stellate cell heterogeneity: Functional aspects and therapeutic implications

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Abstract

Hepatic stellate cells (HSCs) represent a highly heterogeneous mesenchymal cell population with multifaceted roles in liver homeostasis and disease. Beyond their well-established roles in vitamin A storage and scar formation, there is increasing evidence for HSCs in the regulation of hepatic metabolism, regeneration, immunity, and carcinogenesis. Here, we will review the heterogeneity of HSCs in the healthy, aging, and diseased liver, focusing on cell–cell interactions and their contributions to fibrosis, liver zonation and function, regeneration, and cancer. We explore the spatiotemporal diversity of HSCs, highlighting their subpopulations and their specialized roles, as well as the complex bidirectional communications between HSCs, hepatocytes, endothelial cells (ECs), macrophages/monocytes, T cells, and tumor cells. By integrating insights from single-cell sequencing, spatial transcriptomics, preclinical data, and clinical data, this review aims to provide an updated understanding of how HSC heterogeneity contributes to liver homeostasis and disease and can be targeted for therapeutic purposes.

Keywords: bone morphogenetic protein, hepatocellular carcinoma, metabolic dysfunction–associated steatotic liver disease, NAFLD, R-spondin 3

INTRODUCTION

Hepatic stellate cells (HSCs) are liver-resident pericytes, located in the perisinusoidal space of Disse between hepatocytes and the endothelial lining. In the healthy liver, the majority of HSCs are found in a quiescent state and contain characteristic lipid droplets, in which they store about 50%–70% of the body's vitamin A.[1] Upon liver injury, HSCs undergo a well-characterized activation process and transform from their quiescent into a myofibroblastic state, secreting a vast array of extracellular matrix (ECM) proteins.[2] HSCs are the primary fibrogenic cell type of the liver[3–5] and thereby contribute to the development of liver fibrosis, which is the prime determinant of mortality in patients with chronic liver disease.[6–9] Moreover, HSCs also promote the development of hepatocellular carcinoma (HCC), cholangiocarcinoma (CCA), and liver cancer metastasis.[10–13] Contrasting these pathofibrogenic functions, HSCs also exert protective and homeostatic roles in the liver that extend far beyond their well-recognized roles in vitamin A storage[14].

Abbreviations: ALD, alcohol-associated liver disease; BMP, bone morphogenetic protein; CAF, cancer-associated fibroblast; CAR, chimeric antigen receptor; CLD, chronic liver diseases; ECM, extracellular matrix; ECs, endothelial cells; MASLD, metabolic dysfunction–associated steatotic liver disease; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; RSPO3, R-spondin 3; uPAR, urokinase-type plasminogen activator receptor.

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Increasing evidence suggests that HSCs regulate epithelial health, liver regeneration, metabolism, and immunity through communication with neighboring cells, thereby significantly impacting liver health[14]. Recent studies showed that HSCs control the expression of a wide range of metabolic genes and zonation in hepatocytes and protect the liver from metabolic dysfunction–associated steatotic liver disease (MASLD) and alcohol-associated liver disease (ALD) via R-spondin 3 (RSPO3) secretion.[15] Moreover, HSC-derived RSPO3 regulates cyclin D1 expression in midzonal hepatocytes, contributing to the regulation of liver size and regeneration in homeostasis and after loss of functional liver mass.[15,16] HSC-derived HGF protects hepatocytes from liver injury.[14] HSCs communicate with Kupffer cells (KCs) and endothelial cells (ECs) via bone morphogenetic protein (BMP) 9 (encoded by *Gdf2*) and BMP10 to maintain their identity and function, thereby affecting liver zonation and iron metabolism.[17] Furthermore, HSCs have been implicated in the regulation of immune responses, communicating with a wide range of immune cells via cytokines, chemokines, and retinoids, and serving as antigen-presenting cells.[18,19]

To perform these varied and sometimes opposite functions, HSCs show a high degree of diversity and can take on multiple functional states. Single-cell RNA sequencing studies have illuminated a complex picture of HSC heterogeneity, revealing spatiotemporal diversity with distinct subpopulations and functional states in physiological and pathological conditions.[20–26] The first observations on HSC diversity were made by Wake and colleagues in the early 1990s, demonstrating zonal differences in vitamin A HSC lipid droplets.[27] Diversity of activated HSCs was also noted by Magness et al in a transgenic mouse model, where activated HSCs were labeled by αSMA-driven RFP and Col1a1-driven EGFP reporters.[28] Distinct subpopulations of αSMA-RFP+, Col1a1-EGFP+, and double-positive HSCs were observed *in vitro* and *in vivo*, and were characterized by distinct gene expression profiles and specific locations within the liver lobule.[29] Single-cell and spatial transcriptomic analyses extended this concept and revealed zonal gene expression patterns, which not only contribute to HSC diversity but may also shape specific zonal microenvironments and functions through their interactions with other cells.[25,29] In the present review, we will summarize HSC functions in healthy and disease states, focusing on HSC diversity and how the dynamic interactions of HSC subpopulations with other cell types maintain liver health and function in homeostasis and contribute to fibrosis development in the setting of chronic liver disease. Furthermore, we will discuss the concept of restoring the balance between homeostatic and pathogenic HSC subpopulations as a novel therapeutic approach that accounts for the diverse functions of HSCs in the liver.

SPATIOTEMPORAL HETEROGENEITY OF HEPATIC STELLATE CELLS IN THE LIVER

Zonal heterogeneity of HSCs in the healthy state

The liver is spatially and functionally organized into hexagonal liver lobules, which are further divided into 3 zones based on the relative position to the central and portal vein and adaptations to metabolic and oxygen gradients across the lobule.[30] Distinct gene expression in each zone achieves a well-concerted division of labor to maintain key metabolic functions.[30] While zonation is best characterized for hepatocytes and ECs,[31,32] it has become evident that HSCs also show zonation patterns across the hepatic lobule,[24–26,29] which not only contribute to their transcriptomic heterogeneity but also mediate location-dependent distinct functions (Figure 1). The zonal characteristics of HSCs were initially identified through the uneven distribution of vitamin A-storing lipid droplets and the asymmetrical arrangement of the cytoplasmic processes along the hepatic sinusoids, with periportal HSCs containing a higher number of lipid droplets, mostly type II lipid droplets, and pericentral and midzonal HSCs containing fewer and mostly type I lipid droplets (Figure 1).[3,27] It is noteworthy that the quantity, size, and lipid composition of lipid droplets in HSCs are dynamically regulated by vitamin A intake, delivered to the liver via the portal vein.[3,33] Furthermore, periportal HSCs display shorter cytoplasmic processes, whereas pericentral HSCs contain longer cytoplasmic processes with numerous thornlike projections,[3] indicating that pericentral HSCs may be more active in communicating with neighboring cells via direct cell-cell contacts (Figure 1). In addition, pericentral HSCs demonstrate higher levels of BMP signaling and Wnt signaling, whereas periportal HSCs are enriched in genes for matrix adhesion, chemotaxis, PDGF signaling, and NOTCH signaling (Figure 1).[29] In mice, *Rspo3*, *Hhip*, and *Adamtsl2* show a pericentrally to periportally decreasing gradient of expression, whereas *Ngfr* and *Notch3* have the opposite expression pattern. A similar zonal expression of NGFR and ADAMTSL2 was observed in human livers.[25] In human livers, the distribution of $NCAM^{high}$ HSCs corresponds with the locations of nerve fibers that enter the periportal and midzonal areas of the hepatic lobule.[25,34] Furthermore, it has been suggested that a subset of periportal HSCs, labeled by SMMHC-$CreER^{T2}$, do not differentiate into myofibroblasts in CCl_4-induced or high-fat diet–induced fibrosis models but play a role in sinusoidal capillarization based on their close association with CD31 and laminin.[35] How HSC zonation is regulated remains unknown. It is possible that, similar to KCs, which are regulated by gut-derived microbial signals and their gradients,[36] there is some form of auto-organization for HSC zonation, possibly via oxygen or metabolic gradients

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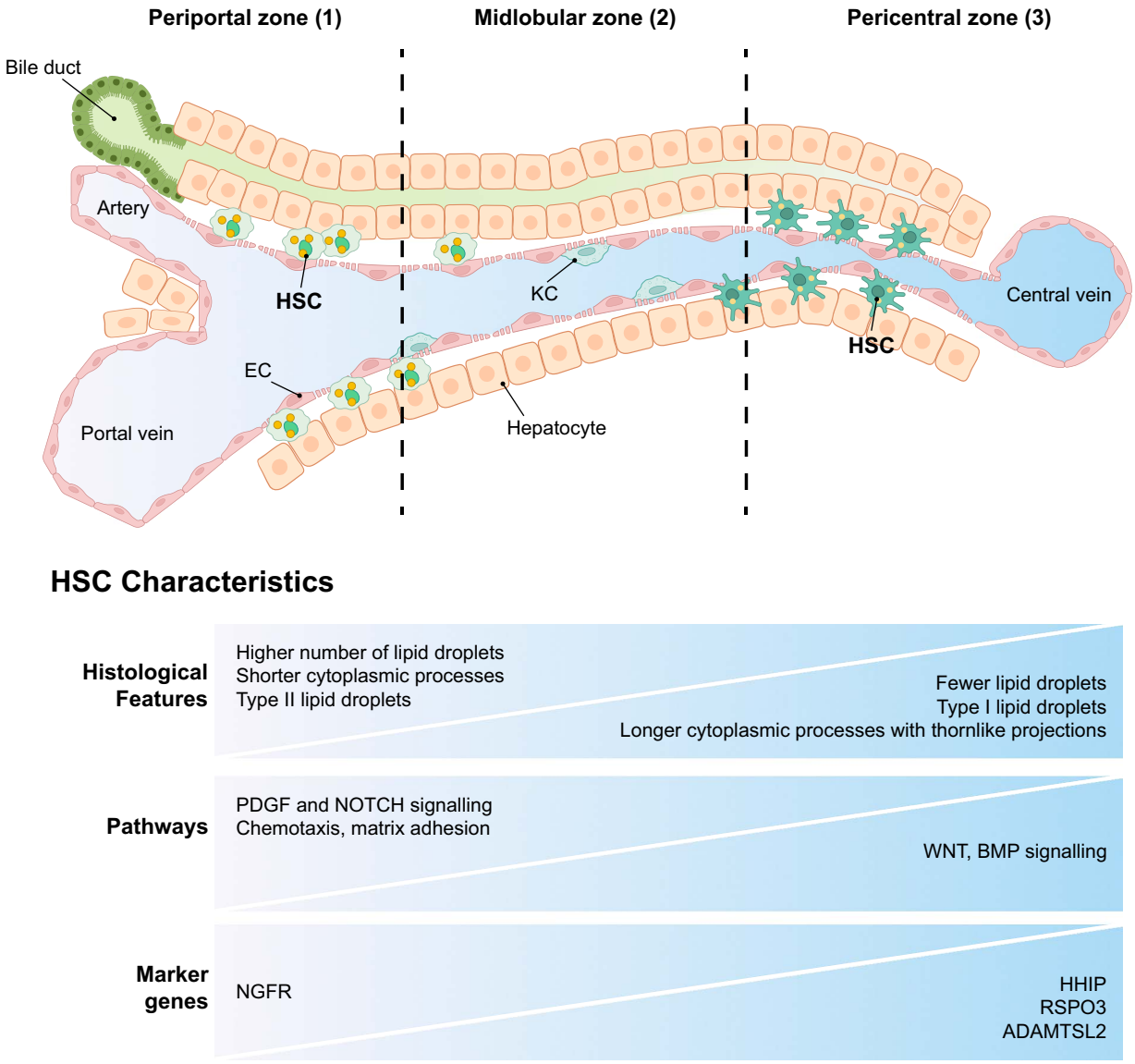

FIGURE 1 Zonal heterogeneity of HSCs. The liver lobule is divided into periportal (zone 1), midlobular (zone 2), and pericentral (zone 3) zones, reflecting anatomic relationships and metabolic and oxygen gradients across the lobule. Periportal HSCs are characterized by a high number of type II lipid droplets and are prevalent with type II lipid droplets, short cytoplasmic processes with few thornlike projections, and high expression of NGFR. Pericentral HSCs typically contain fewer lipid droplets, mostly type I, have longer cytoplasmic processes with more abundant thornlike projections, and are enriched in HHIP, RSPO3, and ADAMTSL2. Pericentral HSCs demonstrate higher levels of BMP signaling and Wnt signaling, whereas periportal HSCs are enriched in matrix adhesion, chemotaxis, PDGF signaling, and NOTCH signaling. Abbreviations: ADAMTSL2, a disintegrin and metalloproteinase with thrombospondin motifs-like 2; BMP, bone morphogenetic protein; EC, endothelial cell; HHIP, hedgehog interacting protein; NGFR, nerve growth factor receptor; NOTCH, neurogenic locus notch homolog proteins; RSPO3, R-spondin 3.

that seem to drive zonation of hepatocytes and ECs.[30,37] Furthermore, the zonation of other cell types, such as ECs, KCs, or hepatocytes, via contact or secreted mediators, may contribute to HSC zonation. For example, pericentral hepatocytes express GDF7, as well as TENM2 and TENM3, which can interact with multiple BMP receptors or ADGRL2 and ADGRL3 on HSCs.[38] Periportal hepatocytes express higher levels of TGFβ3 and PLA2G2A, signaling to nearby HSCs via TGF and integrin receptors, respectively,[38] which may contribute to altered expression of zonation markers such as R-spondin 3.[15] Further increases in the depth and resolution of spatial transcriptomics and integration of other technologies such as spatial metabolomics may allow to characterize HSC diversity and their interactions with neighboring cells more precisely in health and disease.

Temporal HSC heterogeneity in aging and disease

HSC heterogeneity is not solely spatial but also underlies temporal dynamics, with distinct cell states and

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functions evolving over time in health and disease. HSCs can take well-characterized states, which include (i) the quiescent state; (ii) the activated state; (iii) the senescent state; (iv) the deactivated state; and (v) the apoptotic state (Figure 2). While nearly all HSCs remain in the quiescent state in the healthy liver, aging and liver disease lead to increased cell turnover and shuffling between different states, which are associated with altered gene expression and functions. Different from the well-described and distinct states during the maturation of B and T cells, the trajectories of HSCs toward activation, deactivation, and senescence represent continuous gradients (Figure 2). Activated HSCs arise from quiescent HSCs and can either stay in the activated state, revert to a deactivated state,[39,40] become senescent[41] or undergo apoptosis[42] (Figure 2). Replicative senescence and apoptosis are unidirectional trajectories and, therefore, represent terminal states for HSCs (Figure 2). Aging and liver diseases are intertwined as aging constitutes a risk factor for the development and progression of chronic liver diseases.[43–45] *Vice versa*, chronic liver disease may contribute to cellular aging processes that may ultimately end in cellular senescence.[46] Besides changes in hepatocytes and immune cells, there is an increase in the HSC population in aged rodents and humans, with nearly doubled HSC numbers and increased and larger lipid droplets as well as increased HSC activation markers, such as α-SMA and collagen I and matrix remodeling markers.[47,48] During activation, HSCs are not uniformly activated but represent different cell states, which include phenotypes that have been described as intermediately activated HSCs (iaHSC), fully activated myofibroblastic HSC (myHSC), inflammatory and immune HSCs (iHSC), and proliferating HSCs[26,41] (Figure 2). Spatial transcriptomic

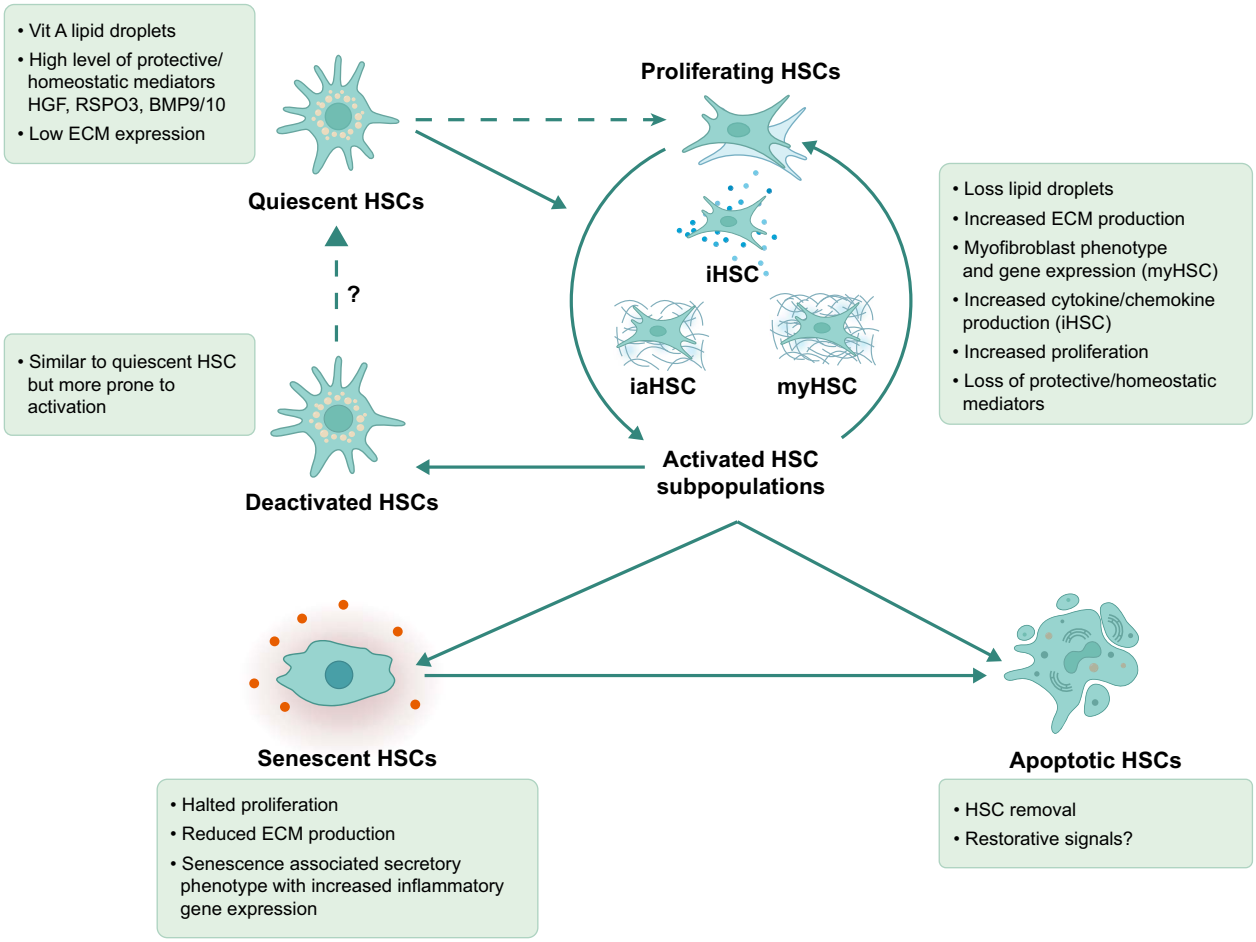

FIGURE 2 Temporal heterogeneity and fates of HSCs. HSCs show distinct cell states with strong changes during disease states and, to a lesser degree, during aging. In the healthy liver, HSCs are mostly in a quiescent state (qHSC). Following liver injury, HSCs activate and can enter phenotypically distinct states. Following activation, HSCs can proliferate to increase the pool of activated HSCs; undergo senescence or apoptosis; or revert to a deactivated state. HSCs represent continuous gradients, and cells can move between different states, with the exception of senescence and apoptosis, which are unidirectional and either preterminal (senescence) or terminal (apoptosis). Activated HSCs display different subpopulations, including myofibroblastic HSCs (myHSCs), intermediate-activated HSCs (iaHSCs), and inflammatory and immune HSCs (iHSCs). Abbreviations: BMP, bone morphogenetic protein; ECM, extracellular matrix; RSPO3, R-spondin 3.

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characterization of mouse and human livers revealed two HSC clusters that were differentiated by the expression of CD74.[38] Studies using fluorescent reporters for αSMA and Col1a1 have found distinct αSMA-positive, Col1a1-positive, and double-positive HSC subpopulations that have unique gene expression and localize to different areas in the lobule, suggesting that activated HSCs may exist as fully blown contractile and ECM-producing myofibroblasts but that there are also populations whose primary function is either ECM production or contraction.[28] Whereas stiffness amplifies HSC activation *in vitro*,[49,50] reduced stiffness, achieved by the deletion of type I collagen, does not significantly reduce markers of HSC activation *in vivo*.[13] Beyond stiffness, ECM viscoelasticity may also regulate HSC activation.[51] Further studies are needed to determine the role and potential location-dependent and zone-dependent effects of mechanical properties, including stiffness and viscoelasticity, in pericentral versus periportal areas or sinusoid versus septal location, on HSC activation *in vivo*.

Like HSCs, HSC-derived cancer-associated fibroblasts (CAF) have been divided into inflammatory CAF (iCAF) and myofibroblastic CAF (myCAF) in the context of liver cancer.[10,11,52] Whereas iCAF and their mediators promote cancer progression,[10,11] the precise functions of iHSC remain to be determined. During CLD, HSCs undergo many rounds of proliferation, which can lead to cellular aging and replicative senescence.[41,53] Senescent HSCs are characterized by increased expression of inflammatory mediators, often termed senescent-associated secretory phenotype, and reduced ECM production.[48,53] Mice with HSC-specific deletion of p53, which decreases replicative senescence, display continuous HSC proliferation, excessive liver fibrosis, and increased development of HCC, suggesting senescence as a mechanism to limit hepatic fibrogenesis and carcinogenesis.[53,54] On the other hand, chimeric antigen receptor (CAR) T cell therapy targeting urokinase-type plasminogen activator receptor (uPAR), designed to remove senescent HSCs, ameliorated liver fibrosis.[55] As uPAR is not specific to senescent HSCs, but is also expressed in activated HSCs and myeloid cells, it is possible that the anti-fibrotic effects of this therapy were caused by the removal of these cells.[41] Besides uPAR, additional genes mark senescent HSCs in mice and humans, including MRC (encoding for CD206).[41] Furthermore, HSCs can undergo cellular fates and thereby enter specific states in CLD that further contribute to HSC diversity. During disease regression, activated HSCs either undergo cell death or revert to a deactivated or inactivated phenotype. Although deactivated HSCs may attain a nearly quiescent state, they appear to retain some memory, most likely epigenetic, and are more prone to activation than their quiescent counterparts.[39,40] *Gabra3* may be a marker of this deactivated HSC population in mice.[26] Furthermore, it has been suggested that deactivated HSCs accumulate in CLD in mice with CCl_4-induced liver fibrosis and patients with MASLD.[56] Finally, HSC turnover in homeostasis and CLD may further contribute to HSC diversity as newly generated HSCs and asymmetric cell division could lead to functionally distinct subpopulations. However, HSC turnover remains poorly understood and, at best, occurs slowly as there is no significant repopulation for several weeks after HSC depletion.[13,16,57] It has been suggested that following recovery of liver injury, newly generated quiescent HSCs from yet-to-be-determined sources, termed recovery-associated qHSCs, may arise and contribute to liver restoration and homeostasis.[58]

HSC heterogeneity in liver disease: Balancing liver function and repair in spatiotemporal contexts

HSCs are the predominant cell type that gives rise to hepatic myofibroblasts and contributes to fibrogenesis.[3–5] It is widely believed that the initial response of HSCs to liver injury aids tissue repair and recovery, whereas persistent injury in CLD leads to a maladaptive process, which is characterized by the continuous activation of HSCs, excessive accumulation of ECM and the progression to fibrosis and cirrhosis.[2,3,59,60] Typically, HSC activation is initiated at the site of injury and subsequently spreads to adjacent areas as the disease progresses. Prime examples are adult MASLD and ALD, where the initial pathophysiology and subsequent fibrosis typically start in the pericentral zone with a perisinusoidal or “chicken wire” pattern, surrounding steatotic and injured hepatocytes and sinusoids.[61–63] With advanced disease, fibrosis may progress toward the portal area with the development of fibrotic septa.[61,64] In contrast, in biliary liver diseases such as primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC), fibrosis originates from the portal area and expands to the lobular area at advanced stages.[65,66] In acetaminophen-induced liver injury, there is a predominant activation of HSCs in the pericentral to midzonal area, where hepatocyte necrosis is typically localized.[24] Accumulating data suggest that HSCs are central components of multicellular and zone-specific signaling circuits that regulate liver function[67] and that disruption of these circuits in CLD affects liver zonation and function[14] and may thereby contribute to the progression of MASLD and ALD[15] and iron overload,[17] which is common in CLD and can accelerate disease progression.[67] In parallel to the loss of homeostatic and protective properties, the increased production and accumulation of ECM in specific areas and over time contribute to major changes in the liver

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microenvironment that alter the behavior of other cell types, in particular hepatocytes, through switches from a physiological to a pathological ECM with increased stiffness and alteration of ECM receptor-mediated signals, leading to loss of hepatocyte function, increased proliferation and increased risk for liver cancer development[13,68] (Figure 3). The most prominent and often zone-specific interactions of HSCs are with hepatocytes, ECs, and KCs and have been reported to affect liver regeneration, MASLD, ALD, iron metabolism, and liver cancer development[13–17] (Figure 3).

Spatiotemporally regulated HSC–hepatocyte interactions in health and disease

In the healthy liver, most cell–cell communication appears to be primarily from HSCs toward hepatocytes, thereby maintaining hepatocyte health and function. As such, HSCs not only store retinoids but also express hepatocyte-protective or hepatocyte-regulatory factors such as HGFand RSPO3, (Figure 3) and possibly FGF18. HGF does not have a known spatial expression pattern in the liver, but there are well-characterized temporal alterations of HGF expression during the

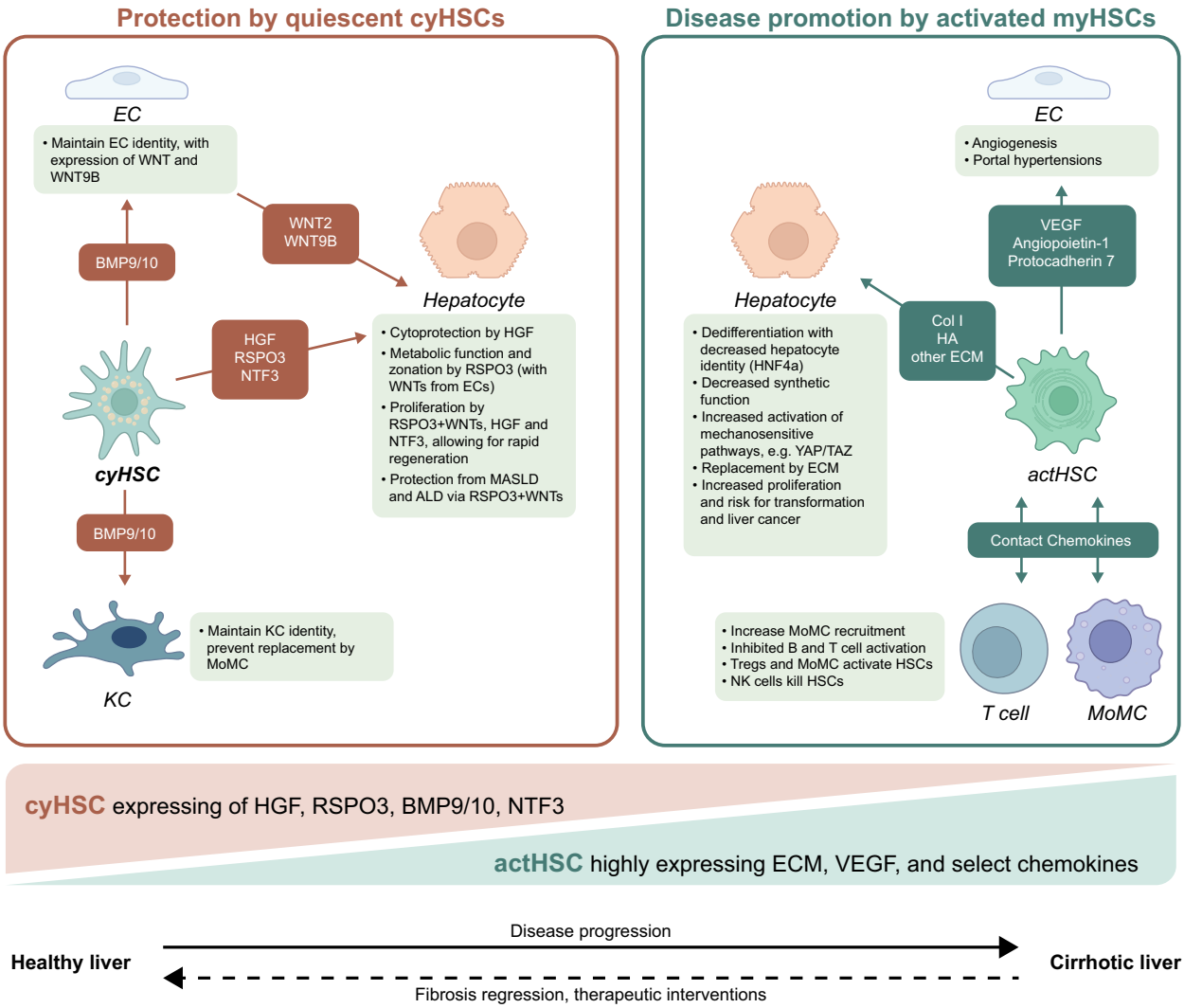

FIGURE 3 Intercellular communication between HSCs and other liver cells. HSCs engage in multifaceted interactions with other liver cell types in health and disease. In the healthy state, these interactions are protective and are mediated by cytokine-secreting HSCs (cyHSCs). CyHSCs mediate hepatocyte homeostasis via HGF and RSPO3, EC homeostasis via BMP9/10, and Kupffer cell homeostasis via BMP9/10. In chronic liver disease, activated HSCs (actHSCs) are pathogenic and produce excessive ECM, which alters hepatocyte identity and function, and increases the risk for malignant transformation and tumor development. Moreover, activated HSCs interact with ECs, promoting angiogenesis and portal hypertension via the secretion of angiogenic factors and the expression of protocadherin-7. Activated HSCs may also increase the recruitment of MoMC and inhibit the activation of T cells through physical contact and via the secretion of chemokines. During chronic liver disease progression, the balance shifts from protective cyHSCs to pathogenic actHSCs. Abbreviations: BMP, bone morphogenetic protein; Col 1, type 1 collagen; EC, endothelial cell; ECM, extracellular matrix; HA, hyaluronic acid; HNF4α, hepatocyte nuclear factor 4-alpha; MoMC, monocyte-derived macrophages; NTF3, neurotrophin-3; RSPO3, R-spondin 3.

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progression of liver disease.[69] In HSCs, HGF expression decreases with activation and disease progression, suggesting the loss of HSC-mediated hepatoprotection over time. Accordingly, mice with HSC-specific HGF deficiency display increased sensitivity to toxic and high-fat diet–induced liver injury.[13] HGF activates its cognate receptor MET on hepatocytes. Accordingly, mice with hepatocyte-specific MET deficiency show similar increases in liver injury and HCC formation as mice with HSC-specific HGF knockout.[70,71] RSPO3 is a central regulator of WNT signaling, which is required for cell surface expression of Frizzled Wnt receptors on target cells. *RSPO3*, one of the prototypical HSC zonation genes,[25,29] shows not only spatial but also temporal expression patterns. *RSPO3* is expressed in pericentral to midzonal HSCs and decreases in mouse and human HSCs during disease progression.[15] Notably, RSPO3 is an important gatekeeper of hepatocyte zonation and function, promoting cytochrome P450 activity, *Aldh2* expression, and cyclin D1 expression.[15] Accordingly, HSC-selective RSPO3 deficiency results in decreased liver size, blunted liver regeneration, increased liver injury in MASLD and ALD, as well as increased tumor formation in MASLD, while decreased CypP450 toxin inactivation (at the benefit of reduced liver toxicity).[15] Combined deletion of Rspo3 and Hgf, which also constitutes a powerful driver of hepatocyte proliferation, exerts potent inhibitory effects on liver regeneration.[15] Neutrotrophin 3 represents another HSC-enriched growth factor and has been shown to promote hepatocyte proliferation.[16] Fibroblast growth factor 18 (FGF18) is enriched in HSCs and contributes to antioxidant defenses, and reduces hepatocyte injury after hepatic ischemia–reperfusion injury (I/R).[72] In contrast to other hepatoprotective HSC mediators, FGF18 becomes upregulated during disease processes. Conversely, hepatocyte-specific deletion of FGF receptor 3 (FGFR3), the primary receptor for FGF18, aggravates liver injury,[73] suggesting FGF18–FGFR3-mediated HSC–hepatocyte communication as another hepatoprotective signaling pathway. Meanwhile, studies have demonstrated that healthy hepatocytes contribute to keeping HSCs in their quiescent state by producing ECM1, which interacts with αv integrin and then stabilizes ECM-deposited TGFβ in its inactive form to prevent HSC activation.[74] Together, these data demonstrate that HSC–hepatocyte crosstalk and high levels of the protective and liver function-promoting HSC mediators such as HGF, RSPO3, and neurotrophin-3 regulate liver homeostasis in a spatiotemporal manner and that the decrease of these homeostatic functions during liver disease progression negatively affects liver function and regeneration while increasing metabolic injury.[14]

In CLD, the progressive loss of homeostatic and protective HSC functions is accompanied by complex cell–cell interactions that contribute to HSC activation, fibrogenesis, and additional feedforward loops that may accelerate disease progression in a spatiotemporally controlled fashion. Damaged hepatocytes trigger HSC activation by inducing inflammation and recruiting macrophages that release profibrogenic mediators such as TGFβ. Moreover, damaged hepatocytes release DAMPs that may directly activate HSCs, such as P2Y14 ligands.[75] HMGB1 is another hepatocyte DAMP that may contribute to HSC activation, as shown in some[76] but not in other studies.[76,77] Many of these cell death-induced signals may be local in the initial stages, activating HSCs in areas of liver injury with specific pericentral, periportal, and pericellular activation patterns. In contrast, other signals, such as TGFβ, seem to have at least some systemic effects, as shown in biliary injury, which may trigger HSC activation signals via TGFβ-SMAD signaling in non-injured lobes.[77] PDGF is a potent mediator of HSC proliferation, survival, and migration.[2] Together, TGFβ and PDGF lead to an expanded pool of activated HSCs, but this process involves a large number of additional mediators, including angiotensin II, leptin, VEGF, Notch and Hedgehog ligands, and immune cell-derived and microbiome-derived signals.[2] However, the spatiotemporal regulation of HSC activation by these specific pathways remains largely unknown. Moreover, during their activation process, HSCs display profound changes in metabolic activity, such as upregulated mitochondrial respiration and glycolysis, which may maintain the higher energetic requirements of aHSCs.[78–85] Thus, it is possible that the metabolic pathways in HSCs contribute to diversity and cell fate decisions such as transition from qHSCs to aHSCs, but possibly also other HSC fates, including inflammatory HSCs (iHSCs), senescent HSCs, and deactivated HSCs. During liver injury, the communication between HSCs and hepatocytes is bidirectional and HSC-derived mediators and ECM proteins, including type I collagen, may contribute to disease progression, either through stiffness[13] or specific receptors such as integrins, DDR1, DDR2, and CD44.[86] A wide range of additional HSC-secreted ECM molecules[87] and other mediators[88] may influence hepatocyte functions in specific locations or disease stages and need further investigation.

Spatiotemporally regulated HSC–EC interactions in health and disease

ECs and HSCs are in close proximity to the hepatic sinusoid and interact closely with each other, as well as in multicellular circuits, to regulate each other's activation state as well as metabolic liver functions and liver health. It is believed that ECs maintain HSCs in a quiescent state in the healthy liver via VEGF-stimulated NO production and that the capillarization of ECs, accompanied by loss of their characteristic

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fenestrations, is permissive for HSC activation.[89] Single-cell RNA-seq analysis in a MASLD mouse model identified an HSC-specific secretome and receptors, which indicated close crosstalk between HSCs and ECs via several ligand–receptor pairs.[90] ECs balance regeneration and fibrogenesis via differential activation of pro-regenerative CXCR7/Id1 versus pro-fibrotic FGFR1/CXCR4 angiocrine pathways.[91] HSCs, on the other hand, also provide important signals to ECs via BMP9 and BMP10, which are critical for EC identity and the expression of Wnt-regulating genes[17,92] (Figure 3). Thus, HSCs and ECs work hand in hand to regulate metabolic liver zonation, with HSC regulating predominantly f Wnt receptor expression on hepatocytes via RSPO3 secretion and ECs regulating Wnt receptor activation via secretion of WNT2 and WNT9B (Figure 3). The multicellular network that regulates liver zonation seems to be ultimately controlled by HSCs via two distinct pathways, that is (i) BMP9/10-mediated control of Wnt expression in ECs and (ii) RSPO3-mediated control of Wnt pathway activity in hepatocytes.[15,17] Further studies are needed to determine spatiotemporal alterations of HSC-expressed BMP9/10 and whether the regulation of EC identity by HSC-derived BMP9/10 occurs during development, in adulthood, or both.

Alterations in angiogenesis and the liver's vascular bed are closely associated with the development of liver fibrosis and cirrhosis.[93,94] Activated HSCs promote angiogenesis in the fibrotic through hypoxia-mediated secretion of VEGF and angiopoietin-1 (Figure 3), which may be induced by HIF1α-, TGFβ-, and Hedgehog-dependent mechanisms.[95–100] Moreover, HSCs contract and contribute to portal hypertension in a protocadherin-7–dependent manner.[18]

In summary, quiescent HSCs seem to maintain a healthy state in ECs and the vascular bed in the normal liver. Activated HSCs, on the other hand, appear to promote angiogenesis in the fibrotic liver as well as in the setting of hepatic carcinogenesis and contribute to vessel contraction and portal hypertension.

Spatiotemporally regulated HSC–immune cell interactions in health and disease

Bi-directional interactions between HSCs and KCs regulate hepatic immunity and fibrogenesis in health and disease. The healthy liver is characterized by a predominance of liver-resident KCs, which are yolk-sac derived.[101] HSCs exert a critical role in maintaining the KC state via BMP9 and BMP10 via their cognate receptor, Alk1, on KCs.[17] Mice with HSC-specific deletion of both BMP9 and BMP10 show a loss of KC identity with reduced expression of KC markers, such as Clec4f and Fabp7, and an increase in monocyte-derived macrophages, demonstrated by increased levels of Cx3cr1 and Ccr2. Importantly, BMP9 and BMP10 expression in HSCs decreases during HSC activation in the context of CLD.[17] Together, these findings suggest that HSC-derived signals are needed to maintain KC state and that the well-established switch from KCs to monocyte-derived macrophages in liver disease is not only due to increased recruitment signals but also the loss of HSC-derived homeostatic signals such as BMP9 and BMP10. Beyond the temporal changes in CLD, further information is needed on the spatial expression of BMP9 and BMP10, as they could contribute to the well-described zonation of KC in addition to microbiome-derived signals.[36]

Vice versa, KC and monocyte-derived macrophages provide essential signals to HSCs that contribute to their activation and survival and thereby promote fibrogenesis. These include TGFβ,[102] the most potent driver of HSC activation, as well as IL-1β and TNFα, which provide survival signals to HSCs.[103] Depletion of KC/macrophages strongly reduces HSC activation and liver fibrosis.[104,105] Efferocytosis of dead hepatocytes by KCs and macrophages has been linked to HSC activation and liver fibrosis.[106] Efferocytosis is a process by which dead cells are removed while suppressing inflammation through the release of TGFβ. TGFβ release during efferocytosis could explain links between hepatocyte death, HSC activation, and liver fibrosis and could cause local activation of HSCs at sites of liver injury.[62] At the same time, failure of or overwhelmed efferocytosis has also been suggested to promote liver fibrosis, as dead hepatocytes may continue to release DAMPs.[106]

There is also crosstalk between HSCs and lymphocytes, affecting adaptive immune responses on the one hand and HSC functions on the other hand. The liver is considered an immune-privileged organ[107] and HSCs have been shown to contribute to immunosuppression via multiple mechanisms. Retinol, stored in HSCs, can be released during HSC activation and converted to retinoic acid, which enhances regulatory T cell (Treg) differentiation and promotes immune tolerance.[108] Moreover, HSCs can directly inhibit the activation and proliferation of naive T cells by an ICAM1-dependent and contact-dependent interaction with T cells.[109] Notably, this inhibitory effect positively correlates with the activation levels of HSCs.[109] HSCs also inhibit T cell-mediated and B cell-mediated immunity through the expression of PD-L1.[110,111] As a source for TGFβ, which exerts strong anti-inflammatory and immunosuppressive effects, HSCs may suppress T cell-mediated immunity in a TGFβ-dependent manner.[18] Furthermore, it was suggested that HSCs may function as antigen-presenting cells and they express both MHC class II and CD11c.[112,113] However, follow-up studies need to further validate their antigen-presenting role as it was reported that HSCs do not cross-present

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antigens.[114] Furthermore, HSCs express innate immune receptors such as TLR4 and TLR9, which promote fibrogenesis but may also alter hepatic inflammation and immunity.[115,116]

Vice versa, interactions of lymphocytes with HSCs can affect fibrogenesis in a positive and negative manner. NK cells may kill HSCs and thereby limit fibrogenesis.[116,117] On the other hand, Tregs can contribute to the activation of HSCs as well as MASH-dependent glucose intolerance via Treg-secreted amphiregulin and EGFR signaling in HSCs.[118]

The roles of HSC subpopulations in carcinogenesis and interactions with tumor cells

Clinical data have identified liver fibrosis, cirrhosis, as well as specific markers of activated HSCs, as risk factors for the development of HCC and CCA.[7,52,119,120] Mouse models of liver cancer have helped to delineate the role of specific HSC subpopulations and their mediators in the development of liver cancer.[52] While the overall effects of HSCs are tumor-promoting in HCC, distinct HSC subpopulations can exert opposing functions on HCC development. Cytokine and growth factor-enriched HSCs (cyHSCs), expressing high levels of HGF, protect from HCC development, whereas collagen-producing and hyaluronic acid-producing myofibroblastic HSCs (myHSCs) promote HCC development.[13] In advanced liver disease, accumulation of HSC-secreted type I collagen generates a stiff environment, leading to the activation of mechanosensitive signaling pathways such as TAZ that trigger hepatocyte proliferation and survival, thereby accelerating the development of HCC.[13] Moreover, type I collagen also activates receptors such as DDR1 to promote proliferation specifically in tumor cells, which express higher levels of DDR1 than their non-transformed counterpart.[13] The changes of ECM stiffness on HCC development are also at the post-deposited stage, with advanced glycation end-products enhancing the matrix viscoelasticity and subsequently promoting tumor cell proliferation and invasion.[121] Furthermore, HSC-enriched growth factors such as Gdf15 and epiregulin may promote the proliferation of tumor cells in HCC,[122,123] but Gdf15 has also been reported to be hepatocyte- and tumor cell-derived.[124,125] Analysis of ligand–receptor interactions[13] and co-implantation studies[126] suggest that direct crosstalk between HSCs and hepatocytes or tumor cells enhances tumor growth and progression. The interactions between HSCs and tumor cells are multifaceted, and HSCs as well as HSC-derived CAF may also contribute to tumor development and progression via reduced immune surveillance via reprogramming of macrophages into a tumor-promoting M2 phenotype,[54] the secretion of MIF,[127] activation of the Sting/IFNy pathway[128] or ECM-mediated exclusion or deactivation of immune cells.[52] CCA is highly desmoplastic, characterized by an abundance of HSC-derived CAF and strong interactions between HSC-CAF and tumor cells, which promote CCA growth as shown by pharmacologic and genetic depletion approaches.[10,129] CCA contains subpopulations of HSC-derived iCAF and myCAF, which both interact with tumor cells to promote HCC growth, but through distinct pathways. iCAF are enriched in HGF and promote tumor cell proliferation, whereas myCAF are enriched in the hyaluronan-producing enzyme Has2, which mediates interactions with multiple cell types via a wide range of receptors.[10] In desmoplastic liver metastasis, HSC-derived CAF also promoted tumor growth and survival and consisted of similar Hgf^{high} iCAF and Has^{high} $Col1a1^{high}$ myCAF.[11] Notably, the effects of HSCs on tumor cells and the outcomes of HSC and tumor cell interaction are highly context-dependent, suggesting that the same regulatory factor may yield divergent outcomes in varying conditions. For example, collagen type I, expressed by myCAFs, promotes HCC via increased stiffness and DDR1[13] but diminishes metastatic tumor growth by acting as a mechanical barrier.[11] Moreover, HGF promotes tumor development in ICC and tumor liver metastasis[11,130] but inhibits HCC development by protecting against hepatocyte injury.[13] Conversely, tumor cells also modulate HSCs in the tumor microenvironment. Hepatoma cells secrete activin B leading to the activation of the tumor-surrounding HSCs, favoring tumor cell metastasis.[45] CTGF, produced by hepatocytes/HCC cells, may act on HSCs, leading to IL-6 release, and STAT3-dependent promotion of tumor growth.[131] Thus, bidirectional interaction shapes HSC and tumor cell heterogeneity in a context-dependent manner and typically leads to the development of a tumor-promoting stromal niche in liver cancer over time.

THERAPEUTIC IMPLICATIONS OF TARGETING STELLATE CELL SUBPOPULATIONS

Fibrotic tissue replaces healthy liver parenchyma, disrupting the normal architecture, impairing blood flow, and affecting the liver's ability to perform essential functions. Accordingly, liver fibrosis contributes to the development of portal hypertension, ascites, gastroesophageal varices, liver failure, and HCC and is a primary determinant of outcomes in patients with chronic liver disease.[6–9] Reducing fibrosis is considered key to improving outcomes in patients with CLD. As primary fibrogenic cells of the liver, HSCs constitute a relevant therapeutic target.[132] At the same time, the aforementioned homeostatic and hepatoprotective functions of HSCs suggest that, in addition to inhibiting the fibrogenic

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functions of HSCs, restoration of the beneficial functions of HSCs may be equally vital in the recovery of liver health.[14] Thus, therapeutic strategies should aim to selectively target or kill pathogenic HSC subpopulations, i.e. activated HSCs, or to restore the balance between pathogenic and homeostatic HSC subpopulations. As TGFβ is not only considered a major driver of fibrogenesis but also a central cytokine that promotes the differentiation of quiescent and thereby homeostatic HSC populations toward a fibropathogenic phenotype, inhibition of TGFβ may represent a powerful strategy to reduce fibrosis while also restoring homeostasis in the liver. However, TGFβ acts on many other cell types, and its therapeutic inhibition is challenging due to major and often systemic side effects such as increased inflammation.[133] Inhibition of TGFβ activation via integrin αv represents a refined strategy that may achieve a more local reduction of TGFβ signals specific to fibrogenic cell types, thereby avoiding unwanted effects on other cell types. HSC-specific deletion of αv integrin and pharmacological blockade of αv-containing integrins have been shown to protect mice from liver fibrosis.[134,135] Moreover, pharmacologic or genetic inhibition of integrin αvβ6 significantly reduces biliary fibrosis after bile duct ligation.[136] Among them, bexotegrast (PLN-74809),[137] a dual αvβ6/αvβ1 integrin inhibitor, is being evaluated in patients with liver fibrosis (NCT04480840). A wide range of additional TGFβ-targeting therapies is currently in development.[133] Killing of pathogenic HSC subpopulations represents another therapeutic option and has been validated by CAR T cell-mediated elimination of FAP-positive HSCs in mice (unpublished data, Yashaswini et al, 2025.) HSC-specific targeting of collagen-chaperone HSP47 via retinoid-conjugated lipid nanoparticles may not only decrease collagen production but also trigger HSC death.[138] In a phase 2 clinical trial, HSC-specific targeting of HSP47 by BMS-986263 was well tolerated and improved METAVIR and Ishak score in patients with cured HCV infection and F3–4 fibrosis.[139] However, a clinical trial (NCT04267393) evaluating the effects of BMS-986263 in MASLD has been terminated. Eliminating senescent HSCs via senolytics might represent another strategy for the treatment of liver fibrosis. Senolytic therapy using CAR T cells, targeting uPAR-positive senescent cells, reduced CCl_4-induced or diet-induced liver fibrosis along with improved liver injury and also improved age-related metabolic dysfunction.[55,140] However, it remains to be defined which uPAR-expressing cell types were eliminated in these studies, as myeloid cells, key instigators of liver fibrosis, also express uPAR.[41] A better understanding of restorative signals that revert HSCs to an inactive phenotype or help HSCs to maintain a high expression of homeostatic and protective mediators, such as HGF, RSPO3, BMP9, and BMP10, may further aid in developing novel therapeutic concepts that seek to restore liver health rather than only reducing fibrosis.

CONCLUSIONS AND FUTURE DIRECTIONS

HSCs demonstrate a high degree of spatiotemporal heterogeneity, governed by their zonal distribution, interactions with surrounding cells, responses to local and systemic signals, as well as aging and disease processes with intermittent phases of progression and resolution that shift cell fates and states. Our understanding of HSC heterogeneity and functionally distinct subpopulations has critically expanded with single-cell RNA-seq-based analyses, spatial transcriptomics, and Cre-lox–mediated knockout of relevant candidates in HSCs. However, significant gaps remain in comprehensively defining the precise functions of these subpopulations in different diseases, particularly in human contexts and outcomes. Moreover, the ability of HSCs to transition between different phenotypic states in response to intrinsic signals or external stimuli raises the opportunity to develop therapeutic interventions that target pathogenic subpopulations while restoring protective and homeostatic subpopulations. Therefore, it is important to identify the key regulatory pathways and mediators that govern these transitions. Moreover, deeper insights into the communication with other liver cell types will provide a more comprehensive understanding of the broader regulatory and mostly multicellular networks that govern liver health and disease. Together, this will allow unlocking novel therapeutic opportunities to shift the balance of HSCs or multicellular units or circuits, consisting of HSCs and their interaction partners, from fibrogenesis to homeostasis, regeneration, or hepatoprotection.

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CONFLICTS OF INTEREST

The authors have no conflicts to report.

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